

Structural Activity of Bovidic Acid and Related Compounds as Feeding Deterrents against *Aedes aegypti*

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ABSTRACT Natural products present in preferred and non-preferred animal hosts of biting insects can exhibit interesting semiochemical properties. Recently, a novel fatty acid compound that acts as a feeding deterrent for blood sucking insects was identified from the gaur (*Bos frontalis*). In our laboratory we have prepared analogues of this novel fatty acid and explored their structure activity relationships to understand the molecular and stereochemical properties that may be responsible for the observed repellent or deterrent effect of these compounds. These hydroxy furanoid fatty acid analogues were evaluated against *Aedes aegypti* (L.) mosquitoes and results indicate that this may generate class of topical repellents for use against insects that transmit pathogens to humans.

KEY WORDS: Bovidic acid, feeding deterrents, *Aedes aegypti*, hydroxy furanoid acid

INTRODUCTION

Numerous pathogens of both man and livestock are transmitted by mosquitoes and other biting arthropods. Repellents have proven to be a reliable means of deterring biting insects. While repellents have been isolated from many naturally occurring plants and animals, very few have been found to occur in Bovids. One exception to this is the gaur (*Bos frontalis*), a wild ox found in India and other Asian countries, has long had the reputation of being unbothered by biting arthropods. Thus, the compound bovidic acid and the homologous R-hydroxylated 2,5-tetrahydrofuranoid carboxylic acids were named in reference to the family Bovidae (Ishii *et al.*, 2004), since the sole known natural source reside within this family. Numerous fatty acids produced in the mammalian integument are well known as a source of natural products (Nicolaidis, 1974). Functionally, these products may inhibit potential pathogens and/or deter and repel insects,

thereby protecting the host. Few comparative studies of mammalian skin chemistry are concerned with the secretory components of macroscopic glands (Albone *et al.*, 1984).

Many botanicals with hydroxy furanoid, cyclic ketone and lactone as the core structures have been reported to elicit a variety of biological activities (Chauhan and Raina, 2006). Acetogenins and annonins with hydroxy bisfuranoid core structures exhibit potent and diverse biological effects such as pesticidal, antitumor and antimalarial activities (Abe *et al.*, 2005; Isman, 2006). Structure-activity relationships have been evaluated for these compounds (De Pooter *et al.*, 1987; Ichimaru *et al.*, 2005). However, because of limited availability and the rare occurrence of this class of compound in the mammalian skin secretions (Colton and Downing, 1983), the structure-activity evaluation is only possible using synthetically prepared compounds. Because of reduced risk to humans, pets and environment, natu-

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ral product based deterrents and repellents have been actively sought as alternatives to synthetic chemicals.

Recent studies by Oliver *et al.* (2003) and Ishii *et al.* (2004) reported identification and isolation of natural 5-(1-hydroxynonyl)-2-tetrahydrofuran-pentanoic acid (as 18-carbon Bovidic acid) from the pelage and skin of a gaur *B. frontalis*. It was concluded that compounds belonging to this class of hydroxyfuranoid acid were responsible for the arthropod deterrent effects exhibited by oil secreted from the skin and hair of these wild animals. Decades ago, Itô *et al.* (1971) isolated a shorter 16-carbon analog from sheep wool (Fig. 1).

These acids have been examined for their structures and stereochemistry; however, comparative biological activities were not pursued. The compound 5-(1-hydroxynonyl)-2-tetrahydrofuran-pentanoic acid (18-Bovidic acid) was recently reported as a mosquito deterrent present on the hair of gaur and has been evaluated for its efficacy as a repellent (Oliver *et al.*, 2003). In the present study we have evaluated analogs of synthetic bovidic acid, the related hydroxy furanoid acid and their esters to identify structural features and stereochemistry necessary to exhibit feeding deterrent activities against *Aedes aegypti* mosquitoes.

MATERIALS AND METHODS

Mosquitoes

Aedes aegypti (L.) (red eye Liverpool strain) used in the study were obtained from the colony maintained at the Walter Reed Army Institute of Research (WRAIR), Silver Spring, MD. The colonies were likely originated from a colony at the United States Department of Agriculture Laboratory in Gainesville, FL (Rutledge *et al.*, 1978). Insects were reared by feeding the larvae on ground Tetramin™ Tropical Fish-food Flakes (Tetra Sales, Blacksburg, VA) (Gerberg *et al.*, 1994). Mated females (5–15-d-old) were maintained in cages with a photo period of 12:12 (L:D) at 27°C and 80 per cent RH and a cotton pad moistened with 10 per cent aqueous sucrose solution. Forty eight hours before use in bioassays, the nulliparous females had access only to water moistened pads, and 24 h before testing, the moistened pads were also removed.

Chemicals

Synthetic C-18 and C-16 acids (6*S*,9*R*,10*R*) were prepared according to the methods of Oliver *et al.* (2003). We found these acids identical to the material isolated both from gaur pelage and from sheep wool (Ishii *et al.*, 2004). Their enantiomer C-18 and

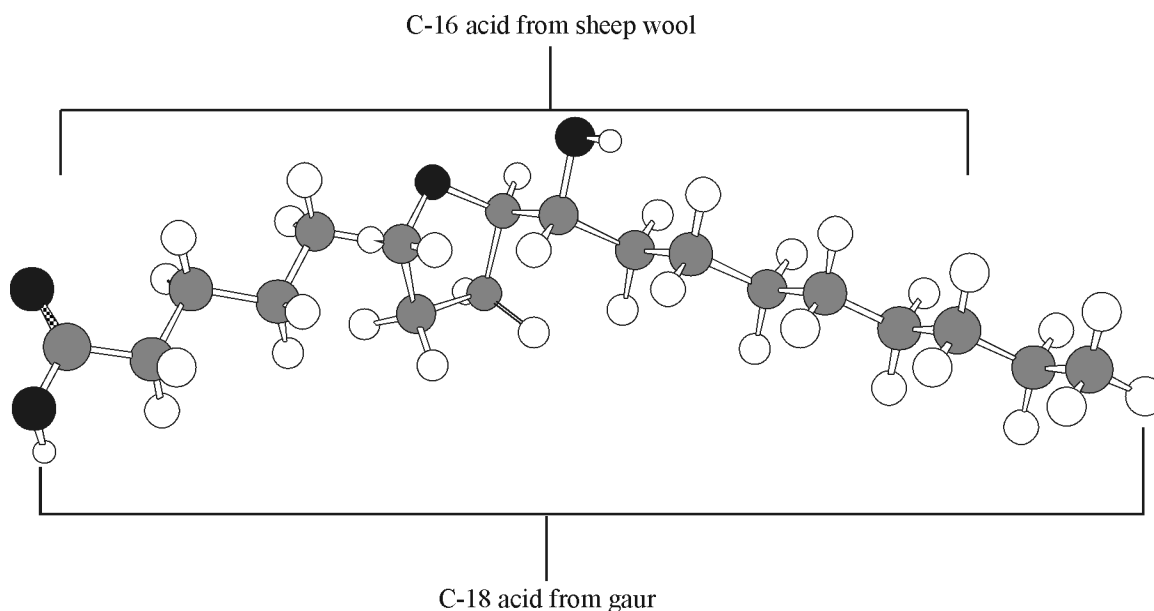


Fig. 1. Structure of bovidic acid.

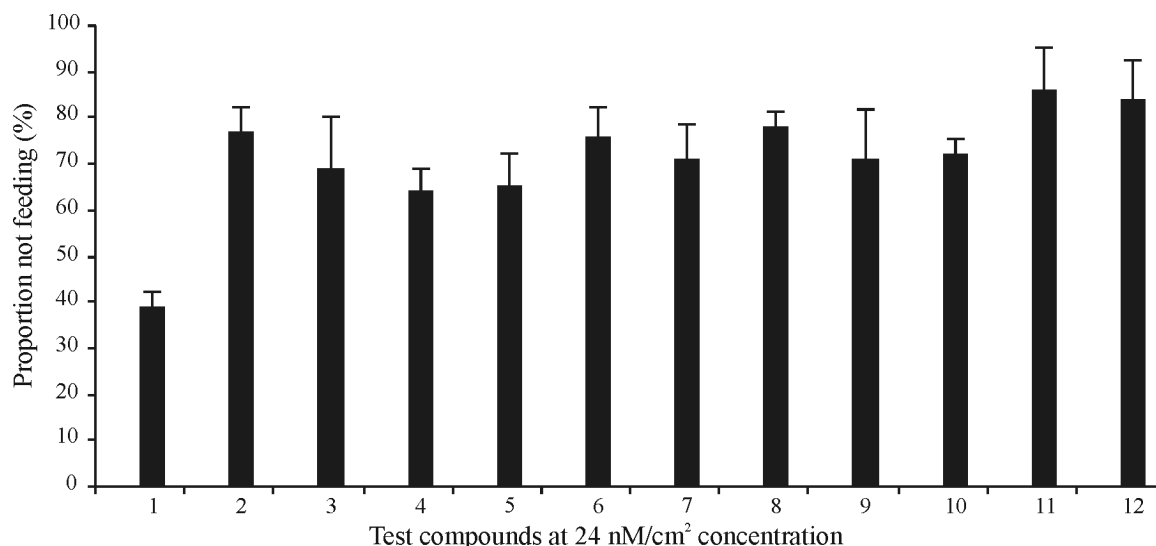


Fig. 2. Proportion not feeding (%) of *Aedes aegypti* females by exposure to feeding in K&D module with test chemicals at 24 nmol/cm² cloth. Compound #1 ethanol, #2 DEET, #3 C-16 acid, #4 C-16 acid (en), #5 C-16 rac-acid, #6 C-18 acid, #7 C-18 acid (en), #8 C-18 rac-acid, #9 C-16 ester, #10 C-16 ester (en), #11 C-18 ester, #12 C-18 ester (en)

C-16 acids (6*R*,9*S*,10*S*) were prepared by following the procedure of Evans *et al.* (2004). Methyl esters of all four hydroxy furanoid acids were prepared by standard esterification procedure. Deet was obtained from Morflex, Inc. (Greensboro, NC 27403). Racemic hydroxy furanoid acids were formulated by mixing 1:1 ratio of (6*S*,9*R*,10*R*) and (6*R*,9*S*,10*S*) acids, and homogeneity was confirmed by GC (HP 6890) equipped with a B-cyclodextrin dimethyl chiral column (0.25 μ m film thickness, 30 m x 0.25 mm ID; J and W Scientific, Folsom, CA) that allowed flame ionization detection (FID) of the compounds. Nitrogen was used as the carrier gas, and the injector temperature was 250°C. The column temperature was 50°C for the first 2 min, rising to 220°C at 2.5°C/min, and then held for 10 min.

Feeding Deterency Assay

The *in vitro* K & D module (Klun *et al.*, 2005) assay system consists of a six-well blood reservoir with each of the 3 x 4 cm wells containing 6 ml human blood cells water-bath warmed (38°C) and covered with a collagen membrane (DeVro Inc., Columbia, SC). The blood-membrane unit simulates a human host for determining mosquito feeding, and anti-biting activity of standard repellent compounds. Activities measured in the system are known to be

comparable to activities observed when tested on the skin of human volunteers. The advantage of this assay is that mosquitoes can be tested much more quickly and without the burden of soliciting human volunteers or concerns about the potential toxicity of compounds being evaluated (Chauhan *et al.*, 2005). Treatments in 110 μ l ethanol were each randomly applied to six 4 x 5 cm areas of organdy cloth (G Street Fabrics, Rockville, MD), and (after air drying) positioned over the membrane-covered blood. K & D modules containing 6 mosquitoes per cell were positioned over the treated cloth and exposed to the treated surface for 3 min. The number of mosquitoes feeding (proboscis inserted through cloth and collagen membrane into blood) in the exposure period was recorded by determining blood engorged females. The compounds used for these tests were applied at 24 nmol/cm² each to organdy cloth because we wished to specifically compare the performance of synthetic bovidic acid and its isomers at a dose that was stoichiometrically equivalent to a dose of deet known to suppress *A. aegypti* biting by at least 80 per cent in a similar testing situation (Klun *et al.*, 2005). At a single dose, the deet, synthetic acids, esters and analogue treatments were replicated 12 times against blood feeding of *A. aegypti*. The comparison of activities was recorded as the pro-

portion of non-feeding mosquitoes of the total used in testing. Because random effects for subject selection is not present for the *in vitro* data, a reduced model with only fixed effects was estimated using Proc Logistic (SAS Institute, Inc., 1999). Statistical comparisons of the compounds were made using Proc Multtest with the step-down permutation p adjustment option to the Cochran-Armitage test.

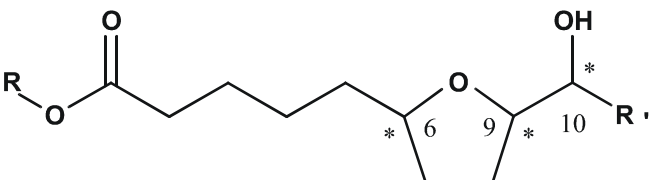
RESULTS AND DISCUSSION

Throughout the *in vitro* bioassay, mosquitoes clearly exhibited fewer landings and feedings in the presence of acids and esters and the positive control Deet, than against the negative control ethanol. The results are presented in Table 1 and depicted as a bar plot in Fig. 2. Our tests demonstrated that both synthetic acids and esters, identical to and similar to that naturally found in gaur hair and sheep wool and their diastereomers or analogues deter contact and feeding by *A. aegypti*. Regardless of whether the side chain is shorter or longer, the effective deterrence of mosquitoes depended on the core hydroxy furanoid structure of the molecule. Table 1 shows the mean \pm standard error proportion of non-

biting mosquitoes. These values were back-transformed to the original scale from the logit scale for ease of interpretation; significance tests were made on the latter scale. At a dose of 24 nmol/cm² cloth, all treatments differed significantly from the control (all adjusted $P < 0.007$, $df = 24.6$, t -test), deet and all synthetic test compounds were statistically indistinguishable from each other (adjusted $P = 1.00$, $df = 24.6$, t -test). However, esters of individual isomers were slightly more effective but not statistically significant as feeding deterrents of *A. aegypti* than deet or the free acids (all adjusted $P = 0.856$, $df = 24.6$, t -test).

These results indicate that absolute configuration of a furanoid ring is less important; however stereoisomers based on any of the chiral centers may induce a difference in feeding deterrence (Grieco *et al.*, 2005). There were no significant differences among the individual hydroxy furanoid acid isomers nor between individual analogues and the racemic mixture, indicating absence of synergistic or antagonist effects. Based on these findings, this class of compounds should be examined further as topical repellents. Studies can then be carried out for the

Table 1. Estimated proportions and their standard errors (SE) of non-biting mosquitoes for assessing *in vitro* biting deterrence of bovidic acid synthetic analogues at 24 nmol/cm² cloth.



Treatment	R	R'	Configuration*	Proportion not biting	SE
1. Ethanol				0.39	0.03
2. DEET				0.77	0.05
3. C-16 acid	H	(CH ₂) ₅ CH ₃	6S,9R,10R	0.69	0.11
4. C-16 acid (en)	H	(CH ₂) ₅ CH ₃	6R,9S,10S	0.64	0.05
5. C-16 rac-acid	H	(CH ₂) ₅ CH ₃	racemic	0.65	0.07
6. C-18 acid	H	(CH ₂) ₇ CH ₃	6S,9R,10R	0.76	0.06
7. C-18 acid (en)	H	(CH ₂) ₇ CH ₃	6R,9S,10S	0.71	0.07
8. C-18 rac-acid	H	(CH ₂) ₇ CH ₃	racemic	0.78	0.03
9. C-16 ester	CH ₃	(CH ₂) ₅ CH ₃	6S,9R,10R	0.71	0.10
10. C-16 ester (en)	CH ₃	(CH ₂) ₅ CH ₃	6R,9S,10S	0.72	0.03
11. C-18 ester	CH ₃	(CH ₂) ₇ CH ₃	6S,9R,10R	0.86	0.09
12. C-18 ester (en)	CH ₃	(CH ₂) ₇ CH ₃	6R,9S,10S	0.84	0.08

16-carbon chain (sheep wool); 18-carbon chain (gaur)

development of commercially viable biobased insect repellents.

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